**Original Article**

# Seroepidemiological Investigation of Lyme Disease and Human Granulocytic Anaplasmosis among People Living in Forest Areas of Eight Provinces in China*

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**Abstract**

**Objective** Lyme disease and Human granulocytic anaplasmosis are tick-borne diseases caused by *Borrelia burgdorferi* and *Anaplasma phagocytophilum* respectively. We have investigated infection and co-infection of the two diseases in the population of forest areas of eight provinces in China by measuring seroprevalence of antibodies against *B. burgdorferi* and *A. phagocytophilum*.

**Methods** Forest areas in 8 provinces were chosen for investigation using whole sampling and questionnaire survey methods. 3 669 serum samples from people in the forest areas were tested for the presence of antibodies by indirect immunofluorescent assay (IFA).

**Results** Seroprevalence against *B. burgdorferi* was 3% to 15% and against *A. phagocytophilum* was 2% to 18% in the study sites in the 8 provinces in China. We also found co-infection of *B. burgdorferi* and *A. phagocytophilum* in 7 of the 8 provinces (the exception being the Miyun area in Beijing). The seroprevalence for both *B. burgdorferi* and *A. phagocytophilum* was significantly higher among people exposed to ticks than among people who were not exposed to ticks.

**Conclusion** We conclude that both pathogens are endemic in the forest areas in the eight provinces, but the prevalence of *B. burgdorferi* and *A. phagocytophilum* differs between the provinces.

**Key words:** *Borrelia burgdorferi*; *Anaplasma phagocytophilum*; Co-infection; Residents of forest areas

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## INTRODUCTION

Lyme disease (LD), caused by *Borrelia burgorferi*, is a multisystem illness that primarily affects the skin, nervous system, heart, and joints[1-2]. Human granulocytic anaplasmosis (HGA), caused by *Anaplasma phagocytophilum*, has been recently recognized as an emerging tick-borne disease[3-4]. Patients with HGA often present with nonspecific symptoms that

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include fever, myalgia, headache, chills, lethargy, arthralgia, leukopenia, and hematological abnormalities[5-6].

In China, Lyme disease has been studied since the 1980s. From 1987 to 1996, we carried out investigations on LD in 22 provinces in China[7]. Our investigations showed that LD is widely distributed in China with an average infection rate of 5.06% in the human population. Infection rates of forest people in the Northeast area, Inner Mongolia and Xinjiang Uygur Autonomous Region are over 10%. There are typical LD patients in many areas of China. We isolated more than 100 *B. burgoferi* strains from humans, animals and ticks and demonstrated that there are endemic foci in China. Despite the fact that the causative agent, *A. phagocytophilum*, is widely distributed in ticks and rodents around the country, human infection with HGA has only recently been reported in China[8-9], the first cases being described in Anhui Province in 2008[8]. Seroprevalence of HGA has so far not been investigated in most areas of the country.

In other countries co-infection of Lyme disease with other pathogens such as *A. phagocytophilum* or Babesia has been frequently reported[11-12], but till now there are few reports of co-infection of *B. burgdorferi* with other pathogens in China.

In view of the wide variety of ecological environments and the many kinds of ticks found in China[13], we have investigated the seroprevalence of both Lyme disease and HGA among the human population in forest areas of 8 provinces in China, covering a wide area of the country from north to south.

## MATERIALS AND METHODS

### *Human Population*

We chose forest areas in 8 provinces as investigation sites because of the clear evidence of ticks in these environments. The residents of the investigation sites were recruited by a whole sampling method. Criteria for inclusion were: (1) aged≥5 years, (2) no history of leptospirosis or syphilis, and (3) had lived in the forest areas and often worked in the fields. One or two villages were chosen in each county of each province. The following sites were investigated: Changbai County and Tonghua County in Jilin Province; Huzhu northern forest area and Huangnan Maixiu forest area in Qinghai Province; Diebu County and Minle County in Gansu Province; Yuncheng area and Jiaocheng area in Shanxi Province; Shimen County and Liuyang area in Hunan Province; Daozhen County, Weining County, Songtao County and Jinping County in Guizhou Province; Miyun area in Beijing; Henan oil prospecting bureau in Henan Province.

A questionnaire was designed for the investigation. The information collected included basic personal information, living environment, history of tick bites, work history and clinical symptoms. A total of 3 669 serum samples were collected from 1 398 females and 2 271 males (ranging in age from 5 to 75 years): 10.06% (369/3 669) belonged to the ≤20 age group, 72.80% (2 671/3 669) to the 20-50 age group and 17.14% (629/3 669) to the >50 years age group. All participants provided written informed consent to the study.

### *Serological Tests*

5 mL of venous blood was taken from the elbow vein of each participant in the morning prior to breakfast. Reaction to both anti-*A. phagocytophilum* IgG antibodies and anti-*B. burgdorferi* sensu lato IgG antibodies were detected by indirect immunofluorescence assay (IFA). Commercial kits with HL60 cells infected with a human isolate of *A. phagocytophilum* (Focus Technologies HGE IFA IgG Test Kit, Cypress, CA, USA) were used to test anti-*A. phagocytophilum* IgG antibodies, the titer ≥1/64 being considered as positive according to the manufacturer's instruction. Antigen used to test anti-*B. burgdorferi s.l*. IgG antibodies was prepared from a Chinese human *B. garinii* isolate, PD91. The PD91 isolate was cultivated in BSK-II media at 33 °C for a week, harvested and washed in phosphate-buffered saline. Antigen was spotted onto the wells of microtiter slides and fixed with acetone. A titer of ≥1/128 was considered positive[7].

All serum samples were screened at a concentration of 1/64 with *A. phagocytophilum* and *B. burgdorferi*. Samples reactive with *B. burgdorferi s. l.* at 1/64 were tested at 1:128 and if reactive, at 1/128 were considered positive and not titrated further. Statistical analysis was performed using Pearson's $\chi^2$ test and $P<0.05$ was considered significant.

## RESULTS

### *Seroprevalence of A. phagocytophilum and B. burgdorferi*

Among 3 669 sera tested, 261 were positive against *A. phagocytophilum*, and 379 were positive

against *B. burgdorferi*. Seroprevalence of *A. phagocytophilum* and *B. burgdorferi* differed significantly between the eight provinces (*A. phagocytophilum*: $\chi^2$=124.89, *P*<0.01; *B. burgdorferi*: $\chi^2$=32.73, *P*<0.01) (Table 1, Figure 1, Figure 2).

**Table 1.** Seroprevalence of *A. phagocytophilum* and *B. burgdorferi* among People Who Live in the Forest Areas in 8 Provinces of China

| Areas | Samples | *A. phagocyto-phlum*(%) | *B.burgdo-rferi*(%) |
|---|---|---|---|
| Jilin | 469 | 35 (7.46) | 43 (9.17) |
| Qinghai | 397 | 34 (8.56) | 59 (14.86) |
| Gansu | 386 | 45 (11.66) | 39 (10.10) |
| Shanxi | 397 | 70 (17.63) | 22 (5.54) |
| Hunan | 321 | 23 (7.17) | 27 (8.41) |
| Guizhou | 425 | 18 (4.24) | 59 (13.88) |
| Beijing | 102 | 8 (7.84) | 3 (2.94) |
| Henan | 1 172 | 28 (2.39) | 127 (10.84) |
| Total | 3 669 | 261 (7.11) | 379 (10.33) |

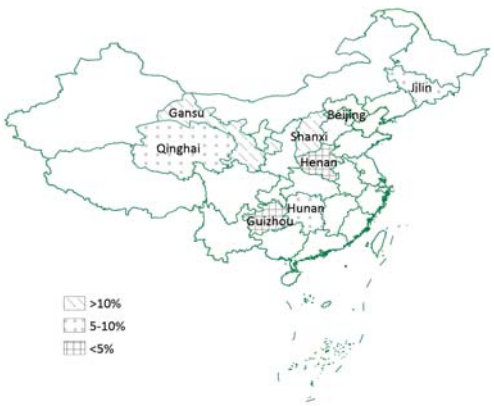


**Figure 1**. The infection rate of AP in eight provinces.

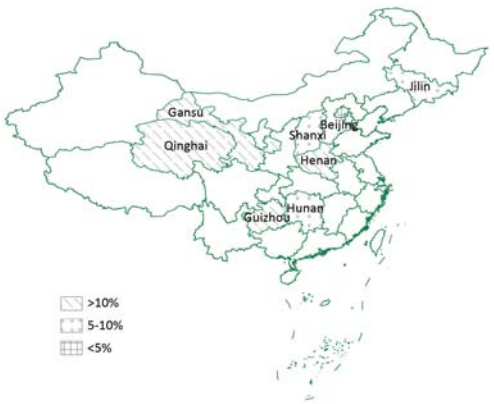


**Figure 2.** The infection rate of Bb in eight provinces.

There were no differences in seroprevalence of antibodies to *A. phagocytophilum* and *B. burgdorferi s. l.* according to sex (*A. phagocytophilum*: $\chi^2$=2.33, *P*>0.05; *B. burgdorferi*: $\chi^2$=0.88, *P*>0.05) (Table 2). The infection rates of *A. phagocytophilum* were different according to age groups ($\chi^2$=19.12, *P*<0.01); however, there was no significant difference in the infection rate of *B. burgdorferi* according to age groups ($\chi^2$=2.14, *P*>0.05) (Table 3).

### *Co-infection of A. phagocytophilum and B. burgdorferi*

Among the sera from 7 of the provinces (excluding Beijing), 46 sera were positive for both *A. phagocytophilum* and *B. burgdorferi* as shown in Table 4.

**Table 2.** Seroprevalence of *A. phagocytophilum* and *B. burgdorferi* of Different Gender Groups

| Sex | Samples | *A. phagocyto-phlum*(%) | *B. burgdo-rferi*(%) |
|---|---|---|---|
| Male | 2 271 | 150 (6.61) | 243 (10.70) |
| Female | 1 398 | 111 (7.94) | 136 (9.73) |
| Total | 3 669 | 261 (7.11) | 379 (10.33) |

**Table 3.** Seroprevalence of *A. phagocytophilum* and *B. burgdorferi* of Different Age Groups

| Age Group | Samples | *A. phagocyto-phlum*(%) | *B. burgdo-rferi*(%) |
|---|---|---|---|
| ≤10 | 108 | 10 (9.26) | 12 (11.11) |
| ~20 | 261 | 28 (10.73) | 21 (8.05) |
| ~30 | 583 | 36 (6.17) | 62 (10.63) |
| ~40 | 1 258 | 76 (6.04) | 137 (10.89) |
| ~50 | 830 | 48 (5.78) | 83 (10.00) |
| >50 | 629 | 63 (10.02) | 64 (10.17) |
| Total | 3 669 | 261 (7.11) | 379 (10.33) |

**Table 4.** Co-infection Rate of *A. phagocytophilum* and *B. burgdorferi* in Eight Provinces

| Study Sites | Samples | Co-infection Samples | Positive Rate (%) |
|---|---|---|---|
| Jilin | 469 | 5 | 1.07 |
| Qinghai | 397 | 18 | 4.53 |
| Gansu | 386 | 10 | 2.59 |
| Shanxi | 397 | 5 | 1.26 |
| Hunan | 321 | 2 | 0.62 |
| Guizhou | 425 | 3 | 0.71 |
| Beijing | 102 | 0 | 0.00 |
| Henan | 1 172 | 3 | 0.26 |
| Total | 3 669 | 46 | 1.25 |

***Infection of People Exposed to Ticks and People Unexposed to Ticks***

404 sera of people exposed to ticks and 182 sera of people unexposed to ticks from Hunan and Shanxi provinces were tested to compare the infection rate of HGA. The infection rate of *A. phagocytophilum* was significantly different between the tick-exposed population (16.58%, 67/404) and the non-tick exposed population (9.89%, 18/182) ($\chi^2$=4.53, $P$<0.05). There was no significant difference between tick-exposed (7.43%, 30/404) and non-tick exposed (3.85%, 7/182) populations for infection rate of *B. burgdorferi* ($\chi^2$=2.72, $P$>0.05).

## DISCUSSION

Both Lyme disease and human granulocytic anaplasmosis are emerging tick-borne zoonosis. Because of similar risk factors and ecologic conditions, infection with *B. burgorferi* and *A. phagocytophilum* can occur at the same time and therefore are often examined together[14-15]. We investigated the prevalence of *A. phagocytophilum* and *B. burgdorferi* infection in people living in forest areas in eight provinces in China. Our data show that both *A. phagocytophilum* infection and *B. burgdorferi* infection occur among people in all the eight provinces, but the positive rates of *A. phagocytophilum* and *B. burgorferi* in eight provinces are very different. The positive rate for *A. phagocytophilum* is similar to the positive rate for *B. burgdorferi* in Gansu, Jilin, and Hunan; Qinghai, Guizhou and Henan have much higher prevalence for *B. burgdorferi* than *A. phagocytophilum*, whereas the prevalence for *A. phagocytophilum* is much higher than *B. burgdorferi* in Beijing and Shanxi. There are many possible reasons for these differences including prevalence of *A. phagocytophilum* and *B. burgdorferi* in ticks and human exposure to ticks. Therefore, further studies are needed to investigate the vectors and reservoirs, and their role in the transmission of *A. phagocytophilum* and *B. burgdorferi* in these areas.

Previous studies show that Lyme disease is endemic in all 8 provinces investigated in this study[7,16-18]. However, the seropositivity rate to *B. burgdorferi* is higher in our study than previous studies in Gansu, Qinghai, and Guizhou. The high prevalence of *B. burgdorferi* in our study may have been because we biased our study towards people living or working in the forest areas. Except for Shanxi, seroprevalence to HGA had not previously been investigated in the other 7 provinces. We showed that seroprevalence to HGA occurs in all 8 provinces investigated. It suggests that HGA is widely distributed in China.

Our investigation also showed co-infection of *A. phagocytophilum* and *B. burgorferi* in seven of the investigated provinces. The co-infection rate was highest in Qinghai (4.53%). Co-infection may affect the process, the severity and the treatment and prognosis of the diseases[19-20]. More attention should therefore be paid to co-infection of *A. phagocytophilum* and *B. burgorferi*.

Our results showed that the infection rate of *A. phagocytophilum* among people exposed to ticks was higher than that of people unexposed to ticks in Hunan and Shanxi. However, there was no significant difference in seropositive rate of antibodies to *B. burgorferi* between the people exposed to ticks and the people unexposed to ticks. The insignificant differences of seroprevalence for Lyme disease among the population exposed to ticks and population unexposed to ticks may be caused by the small size of samples.

The IFA method used in this survey was one of the standard serologic tests for LD, which can provide a useful result but its specificity remains unsatisfactory. It is therefore recommended that positive samples be tested further by other methods for definitive diagnosis of Lyme disease patients[21].

The results of our investigation show that infection with *A. phagocytophilum* and *B. burgdorferi* among people living in the forest areas in China is common. Our study should alert physicians in the investigated areas to the importance of diagnosis and treatment of Lyme disease and HGA.

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